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# The role of CCL22/macrophage-derived chemokine in allergic rhinitis

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**Running Title:** CCL22 in Allergic Rhinitis

**Key Words:** Dendritic Cells, Chemokines, Hypersensitivity, Allergic Rhinitis

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**Abstract**

Dendritic Cells (DCs) are considered to be the most powerful antigen-presenting cells (APCs). DCs are thought to be associated with Th1 or Th2 polarization and with polarization-induced disease such as atopic dermatitis, asthma and allergic rhinitis, but its mechanism is not well known. In this study, we analyzed the mRNA expression of DCs between birch pollen allergic rhinitis and healthy controls by using cDNA array. We found that the expressions of CCL22/macrophage derived chemokine (MDC) differed significantly. We also revealed that CCL22/MDC production was higher in patients than in healthy donors. By chemotaxis assay, CCL22/MDC can enhance the migration of patient's T cells rather than those of healthy controls. Surface marker analysis of migrated cells revealed that the most of migrated cells expressed CCR4, which were considered to be Th2 cells. Furthermore, CD1a<sup>+</sup>CD83<sup>+</sup> cells located in the nasal mucosa expressed CCL22/MDC *in vivo*. To the best of our knowledge, this is the first report clearly indicating the role of CCL22/MDC in allergic rhinitis.

**Key Words:** Dendritic Cells, Chemokines, Hypersensitivity, Allergic rhinitis

## Introduction

Dendritic Cells (DCs) are considered the most powerful antigen-presenting cells (APCs) and play a pivotal role in immune responses [1; 2]. DCs are able to stimulate T cells and to polarize Th1 and Th2 profiles [3]. These two types of helper T-cell responses differ in cytokine production and also in their functional effects. Th1 cells are considered interferon (IFN)- $\gamma$ - producing effector cells that can activate cytotoxic cells and induce cellular immune responses. Th2 cells are considered to secrete interleukin (IL)-4, IL-5, and IL-13, which induce humoral immune responses dominated by an enhancement of immunoglobulin E (IgE) production. In type I allergy such as atopic dermatitis, asthma, and allergic rhinitis, Th2 responses are thought to play a primary role.

Birch pollinosis is a major allergic rhinitis not only in Europe and the Northern part of America but also in Hokkaido, the Northern part of Japan [4]. There have been many reports of birch pollinosis [4; 5] and allergic rhinitis [6], but few have described DCs function and its potential to polarize naive cells to Th1/Th2 cells. By using cDNA array, several hundred gene products can be analyzed in a single experiment [7]. As

such, cDNA array is considered to be suitable for screening mRNAs, with the intent of determining the function of DCs and their effects on immune responses to birch pollen.

In the present study, we used cDNA array technology to investigate the expression of hundreds of genes associated with immune responses to birch pollen. Moreover, we further analyzed gene products that showed different expression between the patients and healthy controls.

## **Materials and Methods**

### *Subjects*

Blood was collected from birch pollinosis patients (n=7) or healthy volunteers as controls (n=5). All pollinosis patients presented seasonal symptoms such as rhinorrhea, nasal obstruction, and sneezing. All patients were diagnosed by nasal examination, nasal smear testing, and a radioallergosorbent test (RAST). Healthy volunteers had no history of allergic disease such as atopic dermatitis, asthma, or allergic rhinitis. All experiments were studied in pollinosis season. Before the experiments, we obtained informed consent from all patients and healthy volunteers.

### *Antigen preparation*

*Betula platyphylla* var. *japonica* was used as whole birch pollen extract. One gram of this material was solubilized in 100 ml of 10 mM phosphate buffer (pH 7.4) at 4°C for 4 hours. After centrifugation (12,000 g, 20 minutes), the supernatant was collected and filtered, then stored at -20°C until use. Endotoxin was not detected in the filtered supernatant (TOXICPLOR®, Seikagaku Corporation, Tokyo, Japan). To determine the amount of protein, the supernatant was measured using a BCA assay kit (Pierce Biotechnology, Rockford, IL).

### *Generation of monocyte-derived DCs*

For cDNA array analysis, peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Conray density gradient centrifugation and were allowed to adhere for 1 h at 37°C in RPMI 1640 (Nissui Pharmaceuticals, Tokyo, Japan) enriched with 10% fetal bovine serum (FBS, Sigma, MO). Adherent monocytes were cultured for 7 days in the presence of interleukin (IL)-4 (50 ng/ml; Peprotech, London, United Kingdom) and granulocyte-monocyte colony-stimulating factor (GM-CSF, 50ng/ml; Peprotech) [8].

For the cytokine assay and migration assay, monocytes were collected by using MACS

CD14<sup>+</sup> microbeads (Miltenyi Biotec, Auburn, CA) and cultured for 7 days in the presence of IL-4 and GM-CSF.

#### *Monocyte-derived DC surface marker analysis*

Cells cultured for 7 days were collected, washed, and incubated at 4°C for 30 minutes with different monoclonal antibodies (mAbs) (all from Immunotech, Marseille, France): fluorescein isothiocyanate (FITC)-conjugated anti-CD14, anti-CD80 or anti-CD83; phycoerythrin-conjugated anti-CD1a, anti-CD40, anti-CD86, anti-HLA-DR. After washing, the cells were analyzed using FACScan (Becton Dickinson, Mountain View, CA).

#### *RNA Isolation*

Generated monocyte-derived DCs were collected, washed twice in RPMI 1640 medium, and resuspended ( $2 \times 10^6$  cell/ml) in a 6-well flat-bottomed culture plate. Resuspended monocyte-derived DCs were pulsed with birch pollen extract (20 µg/ml) and incubated for 1 hour at 37°C. Total RNAs were isolated from pulsed cells using Sepazol reagent (Nakalai Tesque, Tokyo, Japan) according to the manufacturer's protocol. Isolated RNAs were pooled as patients' RNAs and healthy donors' RNAs respectively, because

the amounts of the isolated RNAs were not sufficient for cDNA array analysis.

*cDNA array analysis*

Atlas® Human Hematology/Immunology Array was purchased from Clontech (Palo alto, CA). Probed genes were listed at manufacturer's website

([http://www.clontech.com/upload/images/tools/Atlas%20Arrays/634507\\_HuHemaImm.](http://www.clontech.com/upload/images/tools/Atlas%20Arrays/634507_HuHemaImm.xls)

xls). Reverse transcription-polymerase chain reaction (RT-PCR) was performed on

the RNA samples using a SMART PCR cDNA synthesis kit (Clontech) and an Atlas

SMART Probe Amplification Kit (Clontech). The RT-PCR products were purified,

labeled by <sup>32</sup>P-ATP, and hybridized to the array membranes according to the

manufacturer's manual. The membranes were exposed to a phosphoimaging screen at

room temperature for 30 minutes, and then scanned using a BAS2000 (Fuji Photo Film,

Tokyo, Japan). A grid was applied to images of the hybridization spots, followed by

quantification of the spot intensity using BAStation version 1.31 (Fuji Photo Film)

software. Background signals were defined as the average of the hybridization signals

produced by nine negative controls on the array. All hybridization signals were

normalized to those of actin and glyceraldehyde 3-phosphate dehydrogenase.



*Chemokine ELISA*

Supernatants of  $1 \times 10^6$  generated monocyte-derived DCs pulsed with birch pollen extract (20  $\mu\text{g/ml}$ ) or LPS (10  $\text{ng/ml}$ ) as a positive control were harvested 6, 12, 24, and 48 hours after stimulation. Supernatant without stimulation was used as a negative control. A DuoSet sandwich enzyme-linked immunosorbent assay (ELISA) kit for CCL22 and CCL3 (R&D systems, Minneapolis, MN) was used according to the manufacturer's instructions.

*Chemotaxis Assay*

Generated monocyte-derived DCs were collected, washed twice, and resuspended ( $5 \times 10^4$  cell/ml) in a 24-well flat-bottomed plate. Cells were incubated for 24 hours with birch pollen extract (20  $\mu\text{g/ml}$ ) or LPS (10  $\text{ng/ml}$ ) as positive controls. Cultures without stimulation were used as negative controls. Supernatants of incubated cells were collected and transferred into the lower chemotaxis chamber (24 well 5 $\mu\text{m}$ -pore Transwell™ insert, Costar, Cambridge, MA).  $\text{CD4}^+$  cells, isolated by using MACS  $\text{CD4}^+$  beads (Miltenyi Biotec), were suspended ( $5 \times 10^5$  cell/ml) into the upper chamber and incubated for 24 hours. Transmigrated cells were counted by FACScan for 20 s at

60  $\mu$ l/minute, with gating of the forward and side scatter of the lymphocytes. This counting method was previously described by Bleul et al [9]. The results are shown as a migration index (MI). MI was calculated by dividing the migrated cell numbers with stimulation by those with control culture. In some experiments, anti-CCL22/MDC monoclonal antibody (1 $\mu$ g/ml, DAKO, Glostrup, Denmark) was supplemented in the supernatant of birch pollen extract-pulsed monocyte-derived DCs. A portion of the transmigrated cells was washed twice, incubated for 30 minutes at 4°C with PE-conjugated anti-CCR4 and FITC-conjugated anti-CCR5 (both from BD pharmingen, San Diego, CA), and analyzed using FACScan.

### *Immunohistochemistry*

Surgical specimens of conchotomy (partial resection of turbinate) from allergic patients (n=3) or chronic hypertrophic rhinitis without allergic rhinitis (n=3) were fixed with periodate-lysine-4% paraformaldehyde. Serial frozen sections were used for immunohistochemistry. The antibodies used were as follows: anti-human CCL22/MDC rabbit polyclonal Ab (Peprotech), anti-human CD1a mAb (Immunotech), and anti-human CD83 mAb (Immunotech). The sections were developed with an

Envision plus system (DAKO).

#### *Statistical analysis*

The Mann-Whitney U-test was used for the nonparametric statistical analysis of the chemokine production by monocyte-derived DCs. The Kruskal-Wallis test was used for the chemotaxis assay. Values of  $P \leq 0.05$  were considered statistically significant.

## **Results**

#### *Surface marker analysis of generated monocyte-derived DCs*

After a 7-day culture with IL-4 and GM-CSF, generated cells were analyzed for the expression of surface markers, CD1a, CD14, CD40, CD80, CD86, and HLA-DR (Fig. 1).

1). The cells after cultivation displayed high expression of CD1a, CD40, and CD80, positive expression of HLA-DR, and negative expression of CD14, CD83, and CD86

(Fig. 1). This profile was specific for monocyte-derived immature DCs [6; 8] and

considered to be suitable for cDNA array analysis. There were no differences between patients and healthy controls.

#### *cDNA array analysis*

By cDNA array analysis, many genes showed different expression patterns between

patients and healthy controls (Fig. 2). Table 1 showed the results of significantly high/low genes on the cDNA array. The most different gene expression was CCL22, formerly known as macrophage-derived chemokine (MDC). CCL22/MDC was expressed at approximately 30 times higher levels in patients than in healthy controls (Table 1,2). Because many differences were observed among chemokine mRNA expression, we focused on chemokines for further analysis. Table 2 shows the results of chemokine mRNA expression on the cDNA array. Interestingly, CCL22/MDC was highly expressed in patients, but the expression of CCL17 (thymus and activation-regulated chemokine, TARC) and CCL5 (regulated upon activation, normal T-expressed and secreted, RANTES) between patients and healthy controls were almost the same (patient/healthy control ratios were 1.1 and 0.82 respectively). CCL3 (macrophage inflammatory protein 1- $\alpha$ , MIP-1 $\alpha$ ) and CCL4 (macrophage inflammatory protein-1 $\beta$ , MIP-1 $\beta$ ) were expressed at lower levels in patients than in healthy controls. It is known that the CCL22/MDC receptor is CCR4, which is expressed on Th2 cells, and that the CCL3/MIP-1 $\alpha$  and CCL4/MIP-1 $\beta$  receptor is CCR5, which is expressed on Th1 cells [1; 2]. Furthermore, there is a homology between CCL3/MIP-1 $\alpha$  and

CCL4/MIP-1 $\beta$ . We therefore attempted to quantify CCL22/MDC and CCL3/ MIP-1 $\alpha$  for further study.

#### *Chemokine assay*

CCL22/MDC and CCL3/ MIP-1 $\alpha$  production by cultured monocyte-derived DCs was measured using sandwich ELISA (Fig. 3). The amount of CCL22/MDC was increased both in patients (n=7) and in healthy controls (n=5) after pulsing with birch pollen extract. At 24 hours after stimulation, levels of CCL/MDC in the culture supernatants from patients' monocyte-derived DCs were twice as high as those in healthy controls (p<0.05). In contrast, CCL3/MIP-1 $\alpha$  production in patient DCs was approximately half that in healthy controls after 12, 24, and 48 hours with stimulation (p<0.05). To analyze the specificity of stimulation, chemokine production with LPS was also measured. There were no significant differences observed between patients and donors, except for CCL22/MDC production after 24-hour stimulation with LPS.

#### *Chemotaxis assay*

It is important to determine whether the chemokines produced by DCs affect T-cell migrations. We carried out a chemotaxis assay in an attempt to answer this question

(Fig. 4). Results are shown as a migration index (MI) (Materials and Methods). In patients (open bars) and healthy controls (filled bars), the number of migrated cells increased after LPS or birch pollen extract pulsing. In patients, there were many more migrated cells under birch pollen extract pulsing than with LPS pulsing (MI=2.13 and 1.79 respectively). In healthy controls, there was no difference between LPS pulsing (MI=1.53) and birch pollen extract pulsing (MI=1.49). In addition, anti-CCL22/MDC mAb significantly blocked the migration after birch pollen extract stimulation in patients (MI=1.52), but little effect was observed in healthy controls (MI=1.35). These results indicate that CCL22/MDC plays an important role in T-cell migration in patients, but not in healthy controls. Other chemokines such as CCL3/MIP-1 $\alpha$  in healthy donors may play a role in the migration in healthy controls.

We also analyzed the surface markers of migrated cells in patients (Fig. 5). Migrated cells after birch pollen extract pulsing were primarily CCR4<sup>+</sup> cells (Fig. 5b), which represent Th2 cells. In addition, the number of CCR4<sup>+</sup> cells in the lower chamber decreased with the addition of anti-CCL22/MDC mAb. These results also suggest that CCL22/MDC plays an important role in the migration of T cells, and particularly Th2

cells.

### *Immunohistochemistry*

We showed that CCL22/MDC plays an important role *in vitro*, but it remained unclear whether CCL22/MDC is functional *in vivo*. To clarify this important point, we therefore analyzed the differences in CCL22/MDC expression in nasal mucosa between birch pollinosis and healthy controls. CCL22/MDC was found to be absent in nasal mucosa from healthy controls. Conversely, CCL22/MDC-positive cells were observed in the submucosal region of pollinosis nasal mucosa from patients. In serial sections, these CCL22/MDC-positive cells were mostly subsets of CD1a<sup>+</sup> and CD83<sup>+</sup> cells. These results indicate that mature DCs produce CCL22/MDC in the nasal mucosa of allergic rhinitis, and that CCL22/MDC does play an important role *in vivo*.

## **Discussion**

DCs play a pivotal role in immune responses [1; 2], but the functional process related to this role has remained unclear. We therefore decided to use cDNA array to explore the mechanism of action of DCs. cDNA array allows many types of mRNA expression to

be analyzed in one experiment, and it is therefore the most suitable technique for screening unknown genes and for comparing many types of mRNA expressions [7].

In the present study, we have demonstrated that: 1) there are many differences in mRNA expression between patients and healthy controls; 2) among them, differences in the expression of CCL22, formerly known as macrophage-derived chemokine (MDC), are particularly striking, with the expression in patients being approximately 30 times higher than that in healthy controls; 3) by sandwich ELISA, production of CCL22/MDC in the culture supernatants of patients DCs was found to be twice as high as that of healthy controls; 4) the culture supernatant from birch pollen extract-pulsed DCs enhances the migration of Th2 cells, and anti-CCL22/MDC mAb can block this migration; 5) by immunohistochemistry, the presence of CCL22/MDC was proved in nasal mucosa from patient, but not in that from healthy controls. These findings suggest that CCL22/MDC is a pivotal chemokine in the development of allergic inflammation. To the best of our knowledge, this is the first report clearly indicating the role of CCL22/MDC in allergic rhinitis. In our cDNA array study, isolated RNAs were pooled as patients' RNAs and healthy donors' RNAs respectively, because the



amounts of the isolated RNAs were not sufficient for cDNA array analysis. It is difficult to isolate enough amounts of RNAs from DCs because a large quantity of peripheral blood is needed to generate DCs. This limitation may rise significant bias because of a high beta error. To avoid the error, we analyzed CCL22/MDC expression in various experiments such as ELISA, immunohistochemistry.

In our chemotaxis assay, the culture supernatants from healthy control monocyte-derived DCs were found to enhance migration. Our results also suggest that other chemokines such as CCL17/TARC [10] may play a role in the migration.

CCL22/MDC was first reported as a novel chemoattractant produced by monocytes by Godiska et al [11]. After the first report, Vulcano et al reported that DCs are a major source of CCL22/MDC [12]. CCL22/MDC production is increased by IL-4 [13], which is able to polarize to Th2-type immune responses, and is decreased by IFN- $\gamma$ , which has the ability to polarize to Th1 immune responses.

In allergic disease, CCL22/MDC expression has been reported in epithelium from atopic dermatitis patient [12; 14]. Serum CCL22/MDC levels are also known to be closely related to the disease activity of atopic dermatitis [15] and mycosis

fungoides/Sézary Syndrome [14], which are associated with Th2 polarization.

However, it is reported that CCL22/MDC level in plasma does not change by natural grass pollen challenges [16]. The most likely explanation for this discrepancy between our results and Campbell's results [16] is that difference does not appear with the plasma because DC produces CCL22 locally in a nasal mucosa with the allergic rhinitis patient. In addition, there is little DC in peripheral blood.

CCR4, the receptor of CCL22/MDC [17] and CCL17/TARC [18], is primarily expressed on Th2 cells [19; 20; 21; 22], whereas CCR5, the receptor of CCL3/MIP-1 $\alpha$ , is primarily expressed on Th1 cells [20; 21; 22]. This difference in chemokine receptor expression between Th1 and Th2 cells is useful in classifying transmigrated cells [1; 23]. Accordingly, we analyzed chemokine receptors of migrated cells by using a flow cytometer. Culture supernatants from birch pollen extract antigen pulsed DCs primarily induced the migration of CCR4<sup>+</sup> cells. These results indicate that DCs play a pivotal role in Th2 polarization and the migration of these polarized cells.

Activated Th2 cells produce CCL22/MDC [14], IL-4, and IL-13. IL-4 and IL-13 are able to amplify CCL22/MDC production from Th2 cells [13] and monocytes [19].

Furthermore, CCL22/MDC induces eosinophil chemotaxis, which plays an important role in allergic rhinitis and asthma [24], in a CCR3 and CCR4-independent manner [25].

This circuit is thought to polarize the Th2 response and allergic inflammation.

Methods that could stop this CCL22/MDC-CCR4 circuit would constitute new, powerful, and effective treatment for not only allergic rhinitis, but also atopic dermatitis

and asthma. Gonzalo et al have shown that neutralized anti-mouse CCL22/MDC

antibody prevents lung hyperactivity and a significant reduction of eosinophils [26].

Anti-CCL22/MDC antibody is therefore expected to be a candidate for treatment of nasal hypersensitivity in humans.

In conclusion, we have shown that CCL22/MDC is produced by birch pollen

extract-pulsed DCs and that it attracts Th2 cells. The collected data suggest that

CCL22/MDC plays a pivotal role in allergic rhinitis.

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## Figure Legends

Figure 1. Surface markers of generated DCs.

Monocytes from patients or from healthy controls were cultured in the presence of GM-CSF and IL-4 for 7 days. The cells were collected and analyzed by flow cytometer. ~~Representative data from 5 trials is shown.~~ Results are shown as mean fluorescence intensity (MFI). Error bars mean SEM. *Abbreviation: Ab; antibody.*

Figure 2. mRNA expression analyzed by cDNA array.

Pooled RNA samples were used for the analysis. Some mRNAs were expressed (black circle spot) at higher levels in patients than in healthy controls. CCL22/MDC (open square) was found to be present in patients at 30.9 times higher levels than in healthy donors. The lowest spots of the hybridization membrane were housekeeping genes such as actin.

Figure 3. Measurement of chemokine secreted in the culture supernatants.

CCL22/MDC (A) and CCL3/MIP-1 $\alpha$  (B) secreted into the culture supernatants from



DCs were assayed using sandwich ELISA. Results were corrected by negative control. Open square; birch pollen extract-pulsed DCs from healthy controls (n = 5). Filled square; LPS-stimulated DCs from healthy donors (n = 5). Open circle; birch pollen extract-pulsed DCs from patients (n = 7). Filled circle ; LPS-stimulated DCs from patients (n = 7). Error bars mean SEM. \*P<0.05

Figure 4. Chemotaxis assay.

Migrated CD4<sup>+</sup> cells by birch pollen extract-pulsed DC culture supernatant from patients (open bars, n=5) and healthy controls (filled bars, n=5) were counted three times using FACScan. Results are shown as a migration index (MI), as described in materials and methods. Error bars mean SEM. \*P<0.05

Figure 5. Surface markers of transmigrated CD4<sup>+</sup> cells.

Migrated cells were gated by forward scatter and side scatter, and then analyzed for FITC-conjugated anti-CCR5 (X-axis) and PE-conjugated anti-CCR4(Y-axis). (A) negative control. (B) Migrated cells with supernatant of unpulsed DCs. (C) Migrated

cells with supernatant of birch pollen extract-pulsed DCs. (D) Migrated cells with supernatant of birch pollen extract-pulsed DCs and anti-CCL22/MDC antibody.

Representative data from 5 trials is shown.

Figure 6. Detection of CCL22/MDC in human nasal mucosa by

immunohistochemistry.

In healthy controls, CCL22/MDC was not expressed in nasal mucosa, while CD1a<sup>+</sup> and CD83<sup>+</sup> cells were seen in serial sections. Conversely, in patients, scattered CCL22/MDC positive cells were located in the submucosal region. In nasal mucosa of serial section, CD1a<sup>+</sup> and CD83<sup>+</sup> positive cells also expressed CCL22/MDC.

Representative data from 3 trials is shown, respectively. Original magnifications, x100.

Gene name	Patients	Healthy controls	Patients/ Controls ratio
Significantly high ratio			
CCL22/MDC	578.27	18.71	30.9
CD1B antigen	32.50	3.34	9.73
interleukin 1 receptor, type II	46.51	10.56	4.43
interleukin 4 receptor	35.91	12.16	2.95
interleukin 10	38.98	15.88	2.45
interleukin 8	13.34	5.68	2.35
annexin A2	84.97	43.73	1.94
mal, T cell differentiation protein	107.84	63.15	1.71
CD1C antigen	77.87	50.66	1.54
Significantly low ratio			
L-plastin	5.34	53.31	0.10
CD86 antigen	5.53	58.78	0.09
TYRO3 protein tyrosine kinase	5.29	57.82	0.09
SCYA1 chemokine	3.24	46.99	0.07
EB virus induced gene 2	2.83	56.13	0.05
interleukin 12B	2.58	57.52	0.05
POU domain, class 2	2.40	65.23	0.04
platelet-derived growth factor	0.24	45.48	0.01

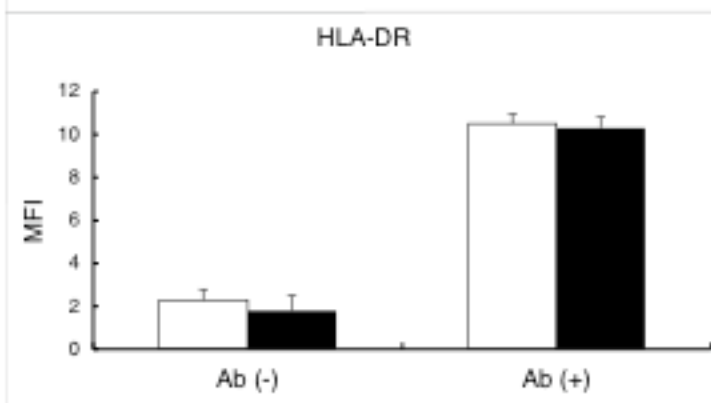
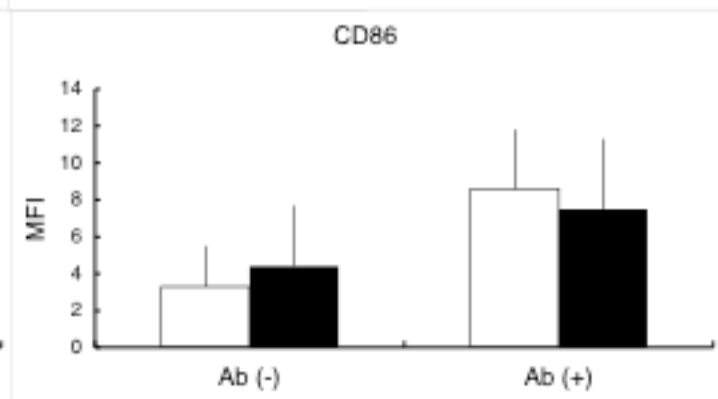
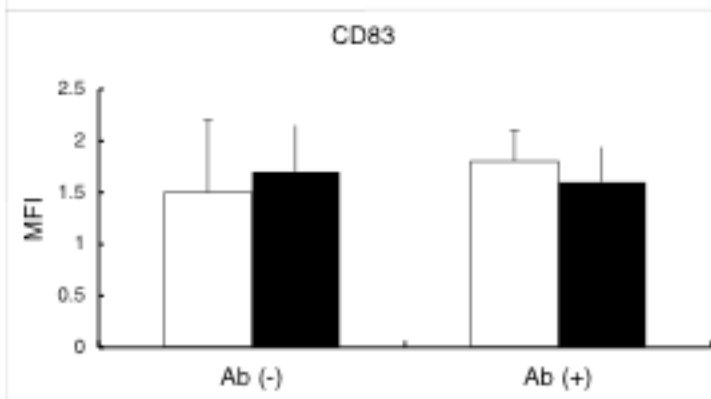
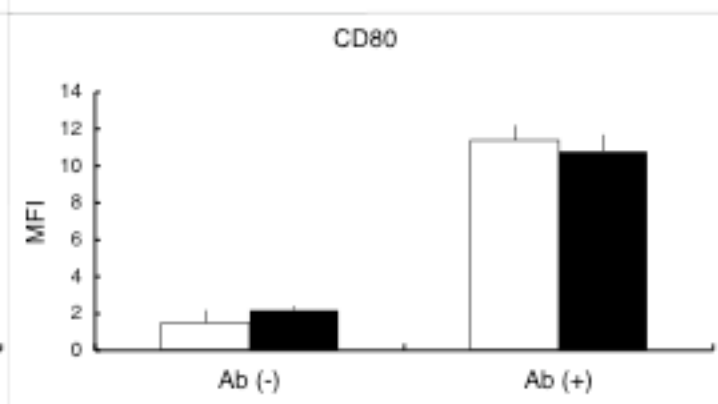
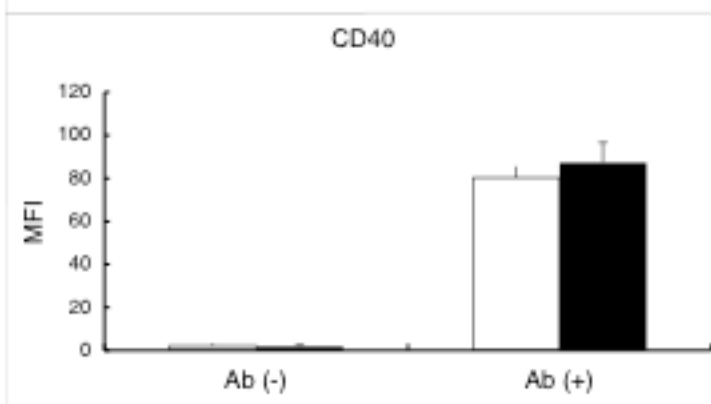
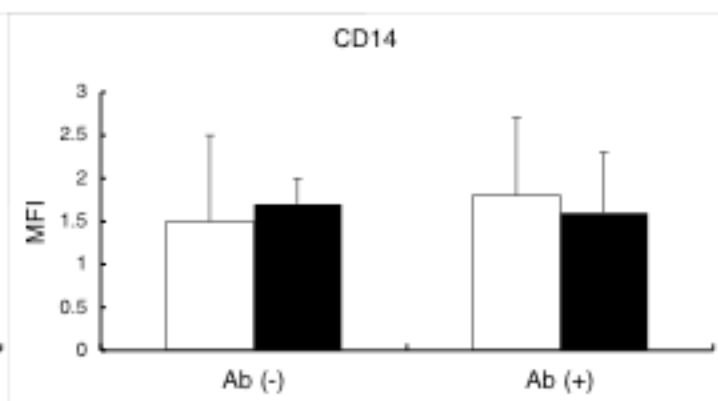
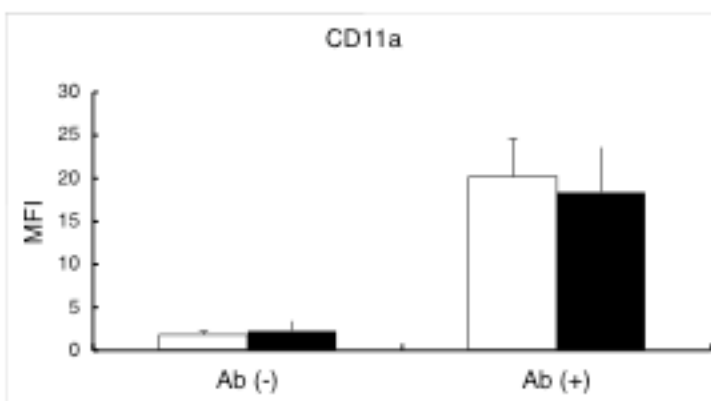
Table 1. Summary of cDNA array assay.

Spot intensity was quantified by using BAS2000 system (Fuji photo film). Intensity was corrected by house keeping genes described in material and methods. Patients/controls ratio was calculated by patients' intensity divided by controls' intensity. Abbreviations: MDC; Macrophage Derived Chemokine, SCYA1; small inducible cytokine A1, EB virus; Epstein-Barr virus.

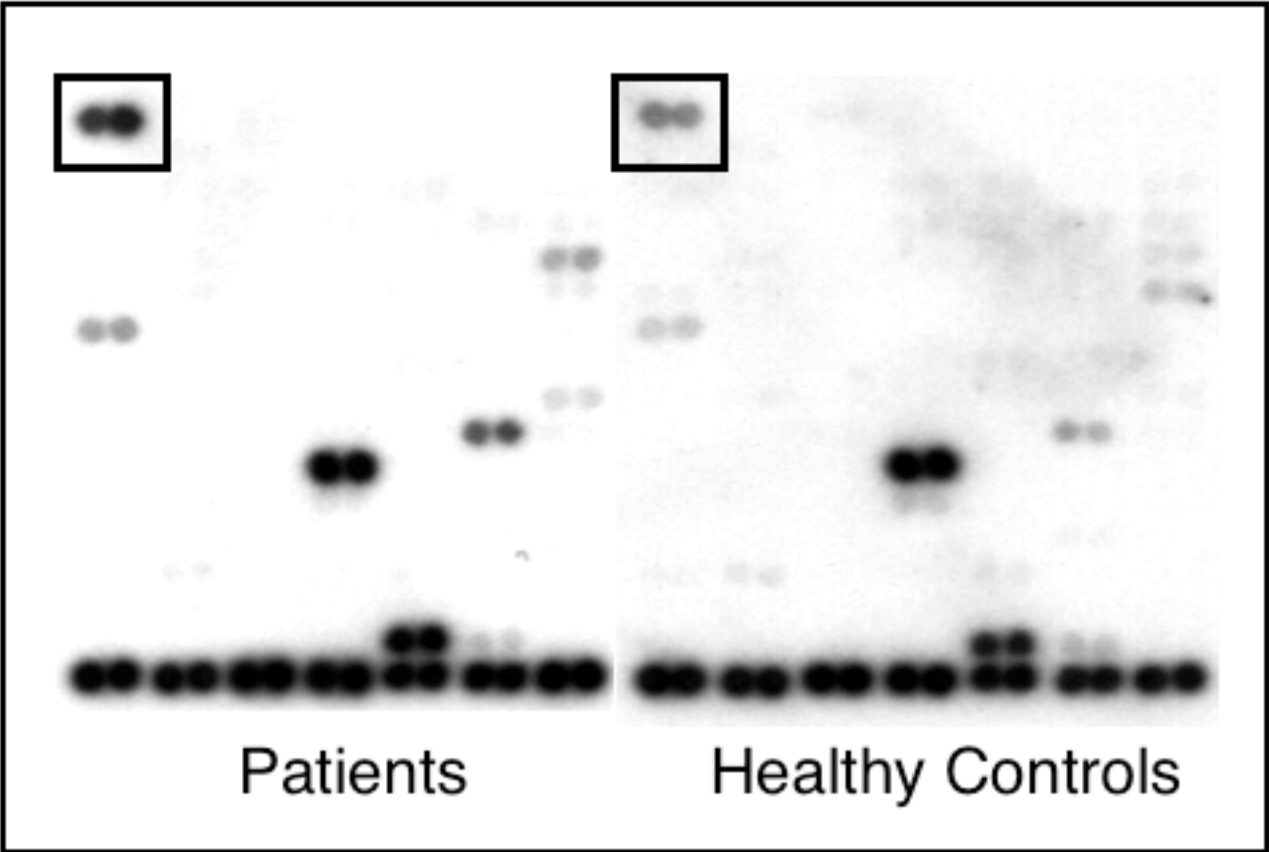
Gene name	Patients	Healthy controls	Patients/ Controls ratio
CCL22/MDC	578.27	18.71	30.97
CCL17/TARC	116.93	105.95	1.1
CCL5/RANTES	959.88	1158.75	0.82
CCL3/MIP-1 $\alpha$	409.63	748.64	0.54
CCL4/MIP-1 $\beta$	624.47	1103.01	0.56

Table 2. Summary of cDNA array assay for chemokines.

Intensity was corrected by house keeping genes described in material and methods. Patients/controls ratio was calculated by patients' intensity divided by controls' intensity. Abbreviations: MDC; Macrophage Derived Chemokine, TARC; Thymus and Activation-Regulated Chemokine, RANTES; Regulated upon Activation, Normal T Expressed and Secreted, MIP-1 $\alpha$ ; Macrophage Inflammatory Protein-1 $\alpha$ , MIP-1 $\beta$ ; Macrophage Inflammatory Protein-1 $\beta$ .

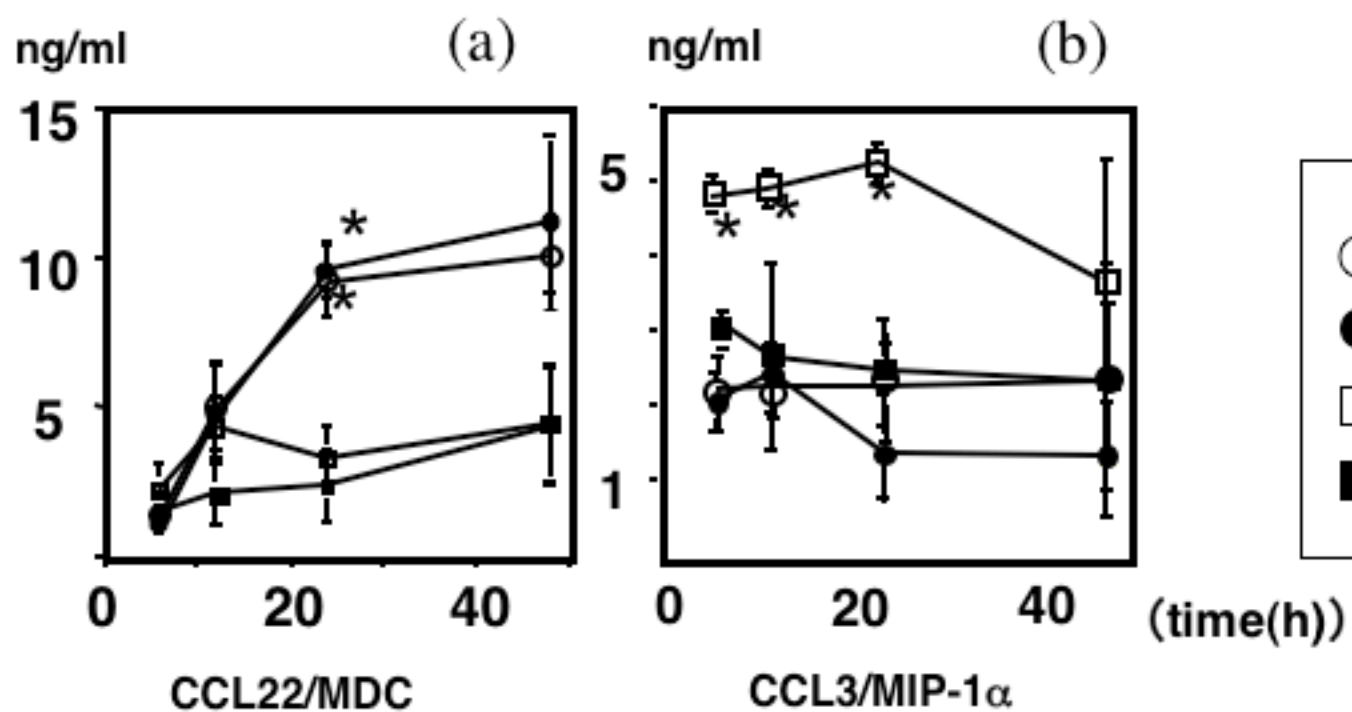


□ Patients (n=5)  
■ Healthy controls (n=5)

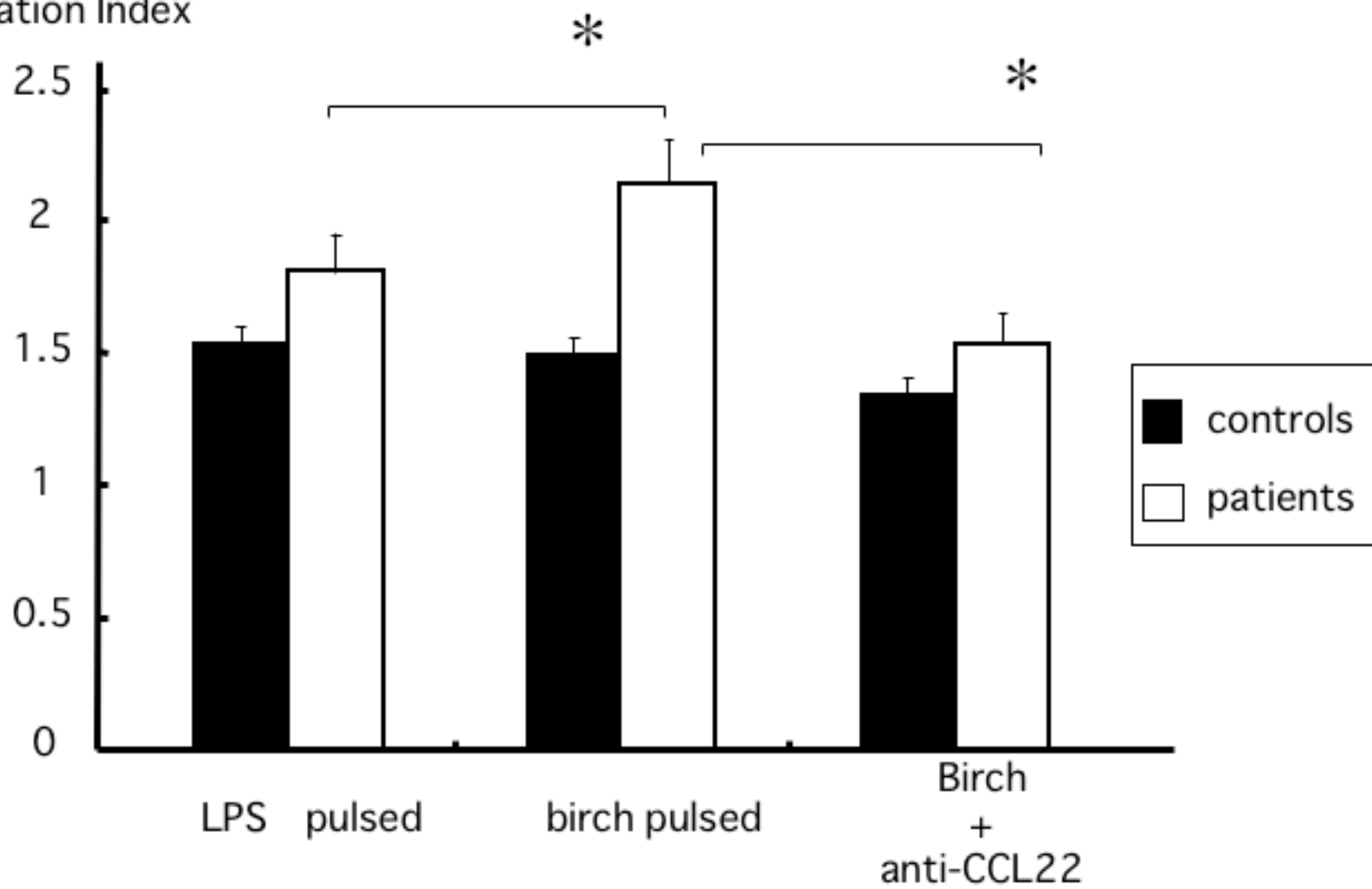


Patients

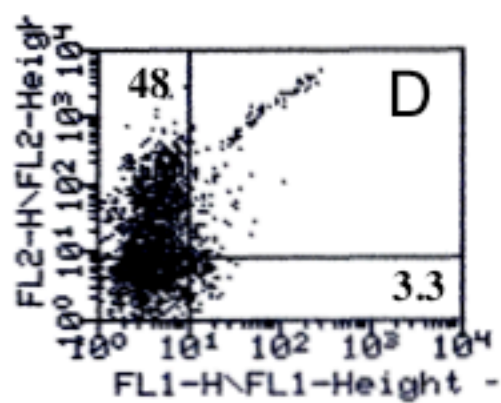
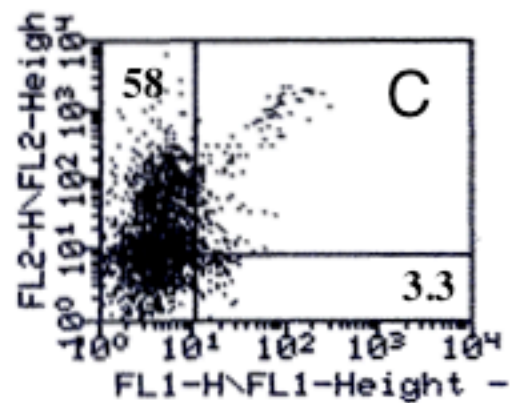
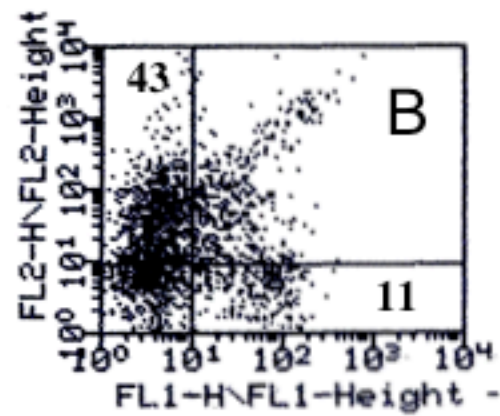
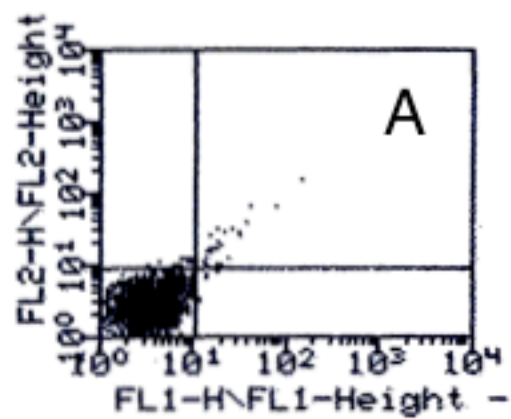
Healthy Controls



Migration Index





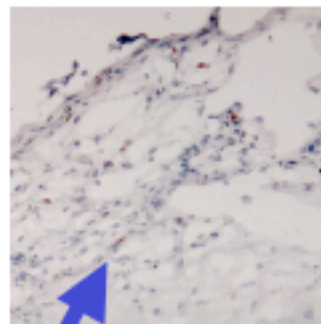
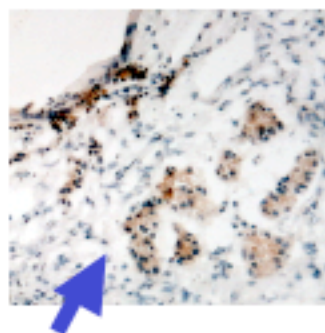
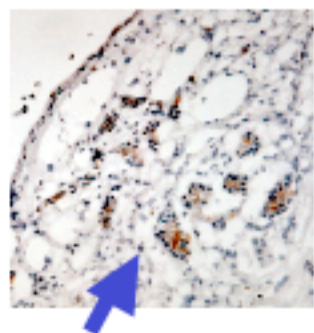


CCL22

CD83

CD1a

Patients



Controls

